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Luciferase of the Scyphozoan Medusa *Periphylla periphylla*

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Abstract. Two types of luciferase that catalyze the luminescent oxidation of coelenterazine were isolated from the marginal exumbrella epithelium (lappet) and the ovary of *Periphylla periphylla;* they were designated luciferase-L and luciferase-O, respectively. Luciferase-L (M_r 32,000), probably derived from highly specialized photocytes, was very resistant to heat, and its activity was little affected by boiling; but it was unstable in solutions of low ionic strength if bovine serum albumin was not included in the solvent. Luciferase-O (M_r 75,000) occurred in the eggs in association with particulate matter, and was solubilized and extracted with a buffer containing 2 *M* guanidine hydrochloride; the enzyme was highly stable in this strongly denaturing solvent. The intensities of the coelenterazine luminescence catalyzed by both luciferases were maximal at pH 7.8 and in the presence of about 1 *M* NaCl. The quantum yield of coelenterazine was estimated to be 0.14 with luciferase-L (emission max. at 465 nm) and 0.12 with luciferase-O (emission max. at 470 nm). The luminescence caused by both luciferases was strongly inhibited by Cu^{2+} and thiol compounds.

Introduction

All three classes of the phylum Cnidaria contain bioluminescent species. In the class Hydrozoa, all reported cases of luminescence are caused by Ca^{2+}-sensitive photoproteins such as aequorin, found in the jellyfish *Aequorea aequorea* (Shimomura *et al.,* 1962), and obelin, obtained from the hydroid *Obelia* sp. (Morin and Hastings, 1971a, b; Campbell, 1974; Visotskii *et al.,* 1989). In the class Anthozoa, light emission is produced by luciferin-luciferase type reactions, such as those of the sea pansy *Renilla* sp. (Cormier, 1978), the sea cactus *Cavernularia obesa,* and the sea pen *Ptilosarcus gruneyi* (Shimomura and Johnson, 1979). The bioluminescence of the class Scyphozoa has never been biochemically studied. In the phylum Ctenophora, many species are bioluminescent, and two of them—*Mnemiopsis* sp. and *Beroë ovata*—contain Ca^{2+}-sensitive photoproteins, mnemiopsin and berovin, respectively (Ward and Seliger, 1974a, b). These proteins are photosensitive and inactivated by exposure to visible light; thus they are distinctly different from the hydrozoan photoproteins.

The luminophore of the photoprotein-based bioluminescence systems of hydrozoans and ctenophores is coelenterazine (Anctil and Shimomura, 1984; Shimomura, 1985). Moreover, this same compound serves as the luciferin (substrate) in the coelenterazine-luciferase system of the luminescent anthozoans (Shimomura and Johnson, 1975; Cormier, 1978). Although the coelenterazine-luciferase system also occurs in many kinds of luminous organisms, including fishes, shrimps, copepods, squids, and coelenterates (Shimomura *et al.,* 1980; McCapra and Hart, 1980; Campbell and Herring, 1990), coelenterazine luciferase has been isolated and investigated in detail from only two of them: *i.e.,* the sea pansy *Renilla* (Matthews *et al.,* 1977) and the decapod shrimp *Oplophorus* (Shimomura *et al.,* 1978).

In a recent study, the luminescence of the scyphozoan jellyfish *Periphylla periphylla* was found to be associated with two distinct sources: one represented by minute, irregularly shaped cytoplasmic granules in the cortical layer of maturing ovarian eggs; the other represented by clusters of even smaller, mostly spherical grains within the cytoplasm of highly specialized photocytes that are

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less than 1 mm apart and distributed throughout the exumbrellar epithelium of the medusae (Herring, 1990; Flood *et al.*, 1996). These photocytes are most concentrated along the margin of the umbrella, which is divided into 16 even-sized lappets by deep groves that lead to the bases of 12 tentacles and 4 sensory organs (Russell, 1970).

We have isolated two luciferases that catalyze the luminescent oxidation of coelenterazine. In correspondence with the findings discussed above, one is from the ovary (luciferase-O) and the other from the lappet (luciferase-L). Although these luciferases are not yet completely pure, they have some extraordinary properties. In the present paper, we report the purification method and some of the properties of these unusual luciferases.

Materials and Methods

Specimens of *Periphylla periphylla* were collected individually, at night, from surface waters of the Lurefjorden north of Bergen, Norway, and placed into 10-liter buckets. The collections were made during December 1995 and March 1996 on board the research vessel *Håkon Mosby* (see Fosså, 1992; Flood *et al.*, 1996; Herring *et al.*, 1996). Lappets and ovarian tissue were cut from the medusae with minimal mechanical disturbance and rapidly frozen at −20°C. After the cruise, the tissues were transferred to a −70°C freezer and were stored at that temperature until used. All experiments were performed at room temperature, except as noted. Bovine serum albumin (BSA) was purchased from Calbiochem (La Jolla, CA); KCl and NaCl were from Fisher Scientific (Pittsburgh, PA); guanidine hydrochloride, Tris, and the protein standards for molecular weight determination were from Sigma (St. Louis, MO).

Measurement of luminescence and luciferase activity

Luminescence intensity and total light were measured with a modified MacNichol-type integrating photometer Model 8020 (Pelagic Electronics, Falmouth, MA) calibrated with the *Cypridina* bioluminescence reaction (Shimomura and Johnson, 1970). The determination of luciferase activity was based on the rate of the light emission caused by the addition to a sample of luciferase (2–50 μl) of 3 ml of 20 m*M* Tris-HCl buffer, pH 7.8, containing 1 *M* NaCl, 0.05% BSA, and 5 μl of 0.2 m*M* methanolic coelenterazine ($A_{430\,nm,\,1\,cm}$ 1.8); exceptions are noted. A full-scale deflection of the meter at the highest sensitivity was defined as one light unit (L.U.) and corresponded to 6×10^8 quanta, except as noted.

Purification of Periphylla *luciferase from lappets*

Lappets (100 g) were thawed and homogenized with a Bamix mixer M 122 (Clark National Products, San Dimas, CA) in 130 ml of 0.05% BSA in water. After its pH was adjusted to 7.2, the homogenate was centrifuged, all at 0°C. Most of the luciferase activity was found in the supernatant, and this activity was purified by four steps of chromatography, as follows. Acetate buffer (10 m*M*, pH 4.8) was used as the basic buffer throughout the purification.

First step. The supernatant (220 ml), having a luminescence activity of 100,000 L.U. (light units), was diluted with 3 volumes of the acetate buffer containing 0.05% BSA, then filtered through a column of Toyopearl SP-650M cation exchanger (Supelco, Bellefonte, PA; 2.5 cm × 7 cm). The luciferase, adsorbed at the top part of the column, was eluted by a stepwise increase (0.1 *M* increments) of KCl concentration, from 0.1 *M* to 0.5 *M* in a pH 4.8 acetate buffer containing 0.05% BSA. The effluent was fractionated.

Second step. The fractions containing luciferase activity (30 ml; 75,000 L.U.) were combined, diluted with 200 ml of the acetate buffer containing 0.05% BSA, and filtered through a column of Toyopearl SP-650M (1.5 cm × 5 cm). The luciferase adsorbed was then eluted by an increasing concentration gradient of KCl, from 0.1 *M* to 0.5 *M*, in an acetate buffer lacking BSA. The luciferase fractions were combined, saturated with ammonium sulfate, and centrifuged.

Third step. The precipitate containing luciferase (55,000 L.U.) was dissolved in 1 ml of the acetate buffer and divided into two parts. Each part was chromatographed by gel filtration on a column of Superdex 200 prep (1 cm × 28 cm; Pharmacia); an acetate buffer containing 1 *M* KCl was used as the solvent.

Fourth step. The combined luciferase fraction (35,000 L.U.) was saturated with ammonium sulfate, then centrifuged. The precipitated luciferase was re-chromatographed on the same column and with the same solvent as used in the third step; the elution curve is shown in Figure 1.

Extraction and purification of Periphylla *luciferase from ovary*

The frozen ovaries (10 g) were thawed and homogenized with about 10 volumes of phosphate buffer (pH 7.0), and centrifuged at 5,000 × *g* for 10 min. The precipitate and floating material were discarded; the cloudy supernatant was saved. The supernatant contained luciferase (500,000 L.U.) in two forms: about 20%–25% was in a dissolved form, and the rest was in an insoluble form that was associated with particulate matter. The supernatant was frozen at −70°C, then thawed and centrifuged at 20,000 × *g* for 20 min. The precipitate that contained the insoluble luciferase was suspended in 5 ml of 10 m*M* Tris-HCl buffer, pH 7.5, containing 0.5 *M* NaCl, and then

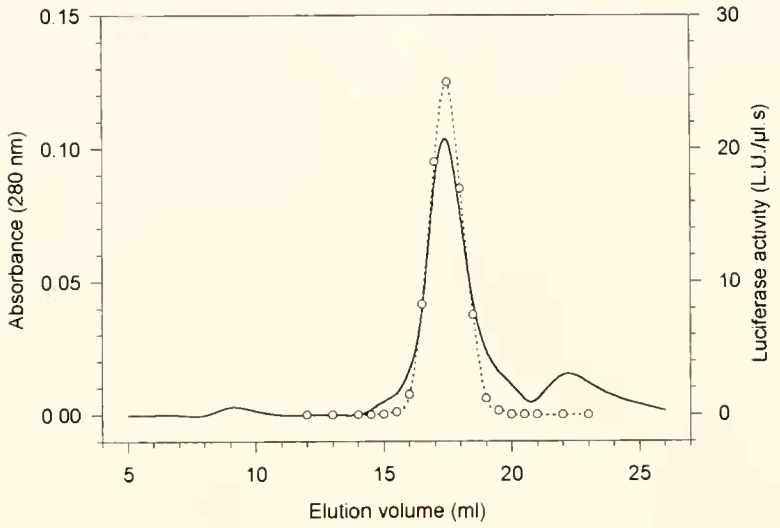

Figure 1. The last step in purification of *Periphylla* luciferase-L on a column of Superdex 200 Prep (1 cm × 28 cm) with 10 m*M* acetate buffer, pH 4.8, containing 1 *M* KCl. Eluent fractions between 16.5 ml and 18 ml were pooled and used as the purified luciferase. Solid line: absorbance measured with a 1-cm light-path cuvette; dotted line: luminescence activity measured at a photometer sensitivity of 3 × 10^9 quanta/L.U.

centrifuged at 20,000 × *g* for 20 min. The supernatant was discarded. The precipitate was mixed with 5 ml of 10 m*M* Tris-HCl buffer, pH 7.7, containing 2 *M* guanidine hydrochloride, 1 *M* KCl and 1% glucose, then sonicated briefly. The mixture, containing a total activity of 350,000 L.U., was centrifuged at 20,000 × *g* for 20 min. The supernatant contained solubilized luciferase (70,000 L.U.), and the precipitate contained the luciferase that remained insoluble (about 200,000 L.U.; not used in the present study). Thus, we estimated that the activity of the insoluble form of luciferase had decreased to about 50% by solubilization. One-fifth of the supernatant (1 ml; 14,000 L.U.) was purified by gel filtration on a column of Superose 6 prep grade (Pharmacia; 1 cm × 19 cm) in 10 m*M* Tris buffer, pH 7.7, containing 2 *M* guanidine hydrochloride, 1 *M* KCl and 0.5% glucose. The elution curve is shown in Figure 2. The gel filtration was repeated four more times to purify the rest of the supernatant.

Results and Discussion

Purification of Periphylla *luciferases*

The luciferase of the lappets (luciferase-L) could easily be extracted with neutral saline solutions. The luciferase activity was sufficiently stable during purification in neutral and acidic media (even at pH 3.0 and below) at 0°C, but was drastically reduced by dilution with water—*i.e.*, by a decrease in the ionic strength. The activity loss was, however, completely prevented, sometimes even reversed, by the addition of 0.05% BSA to the media. The activity of luciferase-L was unstable at a pH higher than 9.0. Thus, the whole process of purification was carried out in a pH 4.8 buffer containing (when appropriate) 1 *M* KCl. In addition, 0.05% BSA was added to the buffer during the cation exchange chromatography as a precaution against inactivation.

The luciferase extracted from the ovary consisted of two types: one was extractable with saline solution and was probably luciferase-L; the other was insoluble in most buffer solutions, even in the presence of 1 *M* KCl, and its activity was associated with tiny particles that were completely retainable on a 0.2-μm filter, but not on a 2-μm filter. The activity of the insoluble enzyme was not easily destroyed by the acidity of pH 1.0, or by a temperature of 90°C. The activity could be extracted from the particles only under very drastic conditions, such as 40% acetonitrile containing 0.2% trifluoroacetic acid; 2% Triton X-100 in 10 m*M* Tris-HCl containing 1 *M* KCl; and 2 *M* guanidine hydrochloride in Tris-HCl buffer containing 1 *M* KCl; but the extraction was partial in all three methods. The last cited solvent was found to be most efficient, solubilizing about 50% of the luciferase activity in one step of extraction; thus, it was used in the present study in the extraction and chromatography of ovarian luciferase (luciferase-O). The relationship between the amount of activity extracted and that retained in the particulate matter indicated that the solubilization causes a decrease of luciferase activity to about half.

The luciferase-L obtained appears to be highly pure on the basis of the chromatogram shown in Figure 1, al-

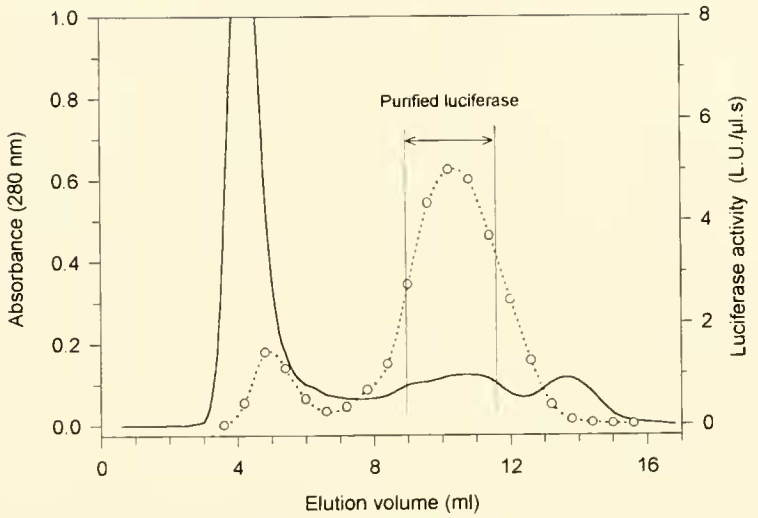

Figure 2. Purification of *Periphylla* luciferase-O by gel filtration on a column of Superose 6 Prep grade (1 cm × 19 cm) using 10 m*M* Tris-HCl buffer, pH 7.7, containing 1 *M* KCl, 2 *M* guanidine hydrochloride, and 0.5% glucose. Solid line: absorbance measured with a 0.5-cm light-path cuvette; dotted line: luminescence activity measured at a photometer sensitivity of 1.1×10^9 quanta/L.U.

though it possibly contained some inactivated luciferase (also see the discussion below in connection with the measurement of quantum yield). The purity of the luciferase-O obtained must be lower than that of the luciferase-L, because the purification method involved only one step of chromatography. The molecular weights of luciferase-L and luciferase-O, as estimated by gel filtration, were about 32,000 and 75,000, respectively (Fig. 3).

Enzymatic properties of the luciferase

The relationship between the intensity of emitted light and the pH of the buffer used (Fig. 4) indicates that

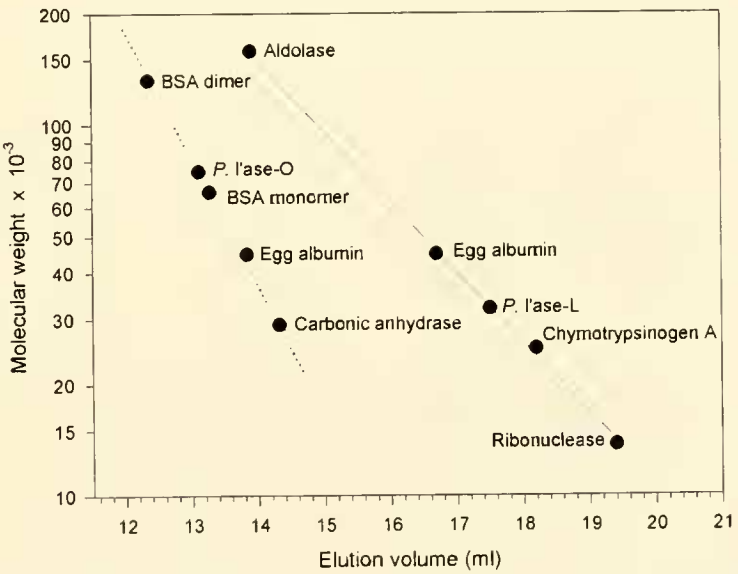

Figure 3. Determination of the molecular weights of luciferase-L (*P.* l'ase-L; solid line) and luciferase-O (*P.* l'ase-O; dotted line) by gel filtration. Luciferase-L was measured on a Superdex 200 Prep column (1 cm × 28 cm) in 10 m*M* acetate buffer, pH 4.8, containing 1 *M* KCl, and luciferase-O on a Superose 6 Prep grade column (1 cm × 21 cm) in 10 m*M* Tris-HCl buffer, pH 7.7, containing 2 *M* guanidine hydrochloride, 1 *M* KCl, and 0.5% glucose. Protein standards used are indicated at appropriate points on the solid and dotted lines.

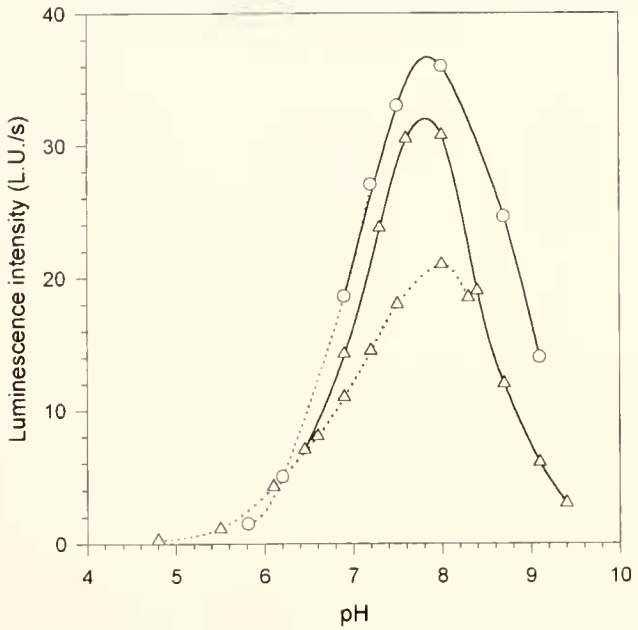

Figure 4. Influence of pH on the intensity of coelenterazine luminescence catalyzed by luciferase-L (○) and luciferase-O (△), in 20 m*M* Tris-HCl buffer containing 1 *M* NaCl (solid lines) and 20 m*M* phosphate buffer containing 1 *M* NaCl (dotted lines); in addition, all solutions used for luciferase-L contained 0.05% BSA.

luminescence intensity is maximal at pH 7.8 for both luciferase-L and luciferase-O. With regard to the effect of salt concentration, the experimental results (Fig. 5) show that luciferase-L has an optimum at about 1 *M* NaCl, whereas the activity of luciferase-O reaches a plateau at about 1 *M* NaCl and stays constant at least up to 3 *M* NaCl. The effect of KCl was essentially the same as that of NaCl. The effect of guanidine hydrochloride was similar when luciferase-O was tested; the optimum concentration was at about 0.6 *M*, indicating that luciferase-O is resistant to this denaturant. In contrast, an increase of guanidine hydrochloride simply decreased the luminescence intensity of luciferase-L, showing no optimum concentration.

The effect of temperature on luciferase-L is highly unusual and intriguing (Fig. 6). The activity of luciferases in catalyzing light emission, *i.e.* luminescence intensity, progressively decreased when the temperature was raised beyond the optimum (about 0°C for luciferase-L; 20°C for luciferase-O), which would be quite normal. In the case of luciferase-L, subsequent cooling of the sample to room temperature (about 25°C) caused an increase of luminescence intensity to the level seen before the temperature was raised. Thus, the inactivation was rapidly reversed upon cooling, so the activity of the luciferase-L was not lost or affected by exposure to the elevated temperature. When a sample was heated at 100°C for 1 min under the conditions described in Figure 6, followed by cooling to room temperature, no loss in the luciferase activity was detected as judged by the intensity of luminescence caused by the addition of coelenterazine; when heated for 15 min at 100°C, the activity decreased by about 20%. Thus, luciferase-L must be a highly thermostable enzyme. The solubilized form of luciferase-O did not have this property, but the insoluble form associated with the particulate matter in the eggs was, like luciferase-L, highly thermostable.

Properties of the luminescence reaction of coelenterazine catalyzed by Periphylla *luciferase*

The spectrum of coelenterazine luminescence catalyzed by luciferase-L (maximum at 465 nm) and that catalyzed by luciferase-O (maximum at 470 nm) are closely similar (Fig. 7). The slight difference found in the emission maxima might indicate that the environment of the coelenterazine binding site is more hydrophobic in luciferase-L molecules than in luciferase-O molecules; this conclusion is based on various data on the emission maximum of aequorin luminescence (Shimomura, 1995a). The slight shoulders seen in the luminescence spectrum measured with purified luciferase-O (550 nm and 620 nm) became much higher and turned into peaks when a less purified preparation was examined (Fig. 7). Probably the peaks were caused by the presence in the sample of unidentified

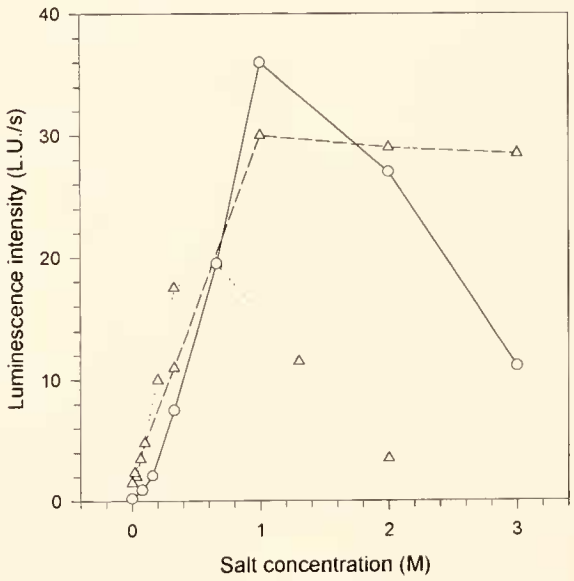

Figure 5. Effect of salt concentration on the intensity of luminescence catalyzed by luciferase-L (○) and luciferase-O (△). Salts used: NaCl, in 20 m*M* Tris-HCl containing 0.05% BSA, pH 7.8 (solid line); NaCl, in 50 m*M* Tris-HCl, pH 7.8 (broken line); guanidine hydrochloride, in 50 m*M* Tris-HCl, pH 7.8 (dotted line).

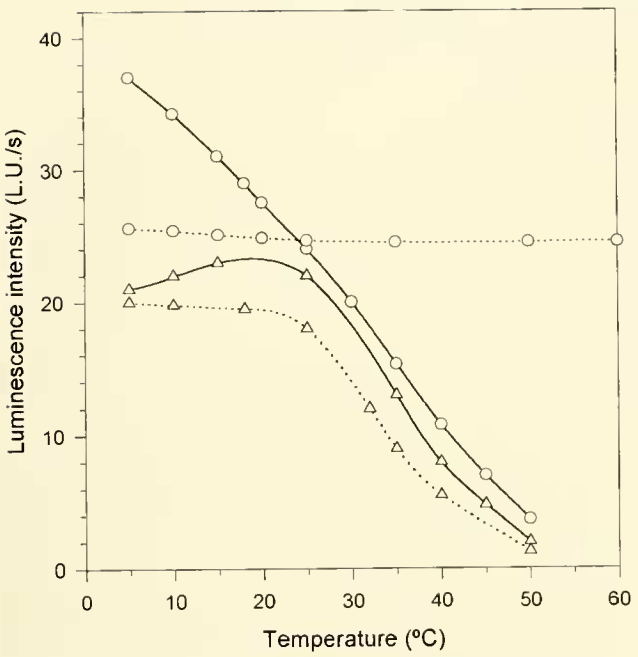

Figure 6. Effect of temperature on the luminescence intensity (solid lines) and the stability of luciferases at various temperatures (dotted lines) in the luminescence of coelenterazine catalyzed by luciferase-L (○) and luciferase-O (△). The luminescence with luciferase-L was measured in 20 m*M* phosphate buffer, pH 7.8, containing 1 *M* NaCl and 0.05% BSA, and the luminescence with luciferase-O was in 10 m*M* phosphate buffer, pH 7.8, containing 1 *M* NaCl. In the measurement of the stability data, the luciferase in the buffer in a test tube was exposed to the indicated temperature for 1 min, then the test tube was equilibrated in a water bath at room temperature for 2–5 min before luminescence was initiated by the addition of coelenterazine.

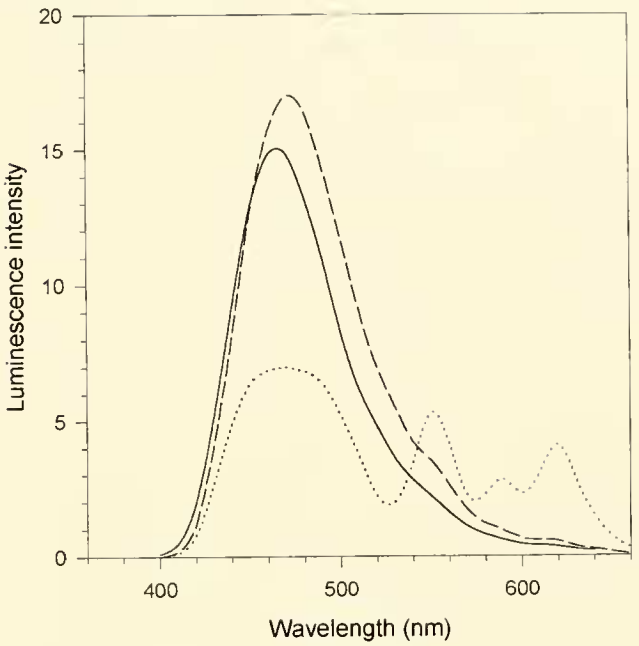

Figure 7. Luminescence spectra of coelenterazine catalyzed by purified *Periphylla* luciferase-L (solid line), purified luciferase-O (broken line), and a crude preparation of luciferase-O (dotted line) in 20 m*M* Tris-HCl buffer, pH 7.8, containing 1 *M* NaCl and 0.05% BSA.

fluorescent substances with corresponding emission maxima. A previously reported observation that the clusters of the grains in the exumbrellar photocytes, but not the grains in the cortex layer of eggs, show a pinkish fluorescence that rapidly fades under UV light (Flood *et al.*, 1996) is apparently not in accord with the present results. One explanation for the discrepancy is that a pink fluorescent substance is more abundant in the lappet photocytes than in the ovarian photocytes, but that the fluorescent substance in lappets easily dissociates from luciferase-L molecules during the extraction process, whereas that in eggs does not dissociate from luciferase-O. In our actual experimental procedure, the lappet fluorescent substance might have dissociated from luciferase-L, even when the frozen lappets were thawed and the cells were broken. In any event, the bioluminescence spectrum of *Periphylla* is probably not affected significantly by these fluorescent substances, because the bioluminescence spectrum of live *Periphylla* was previously reported to be a single peak with a maximum at 470–475 nm (Herring, 1983; Widder *et al.*, 1983).

The luminescence intensity of coelenterazine catalyzed by luciferase was affected by the concentration of coelenterazine up to 3 μM, as shown in Figure 8. From the data in the figure, the Michaelis constant of this enzyme is

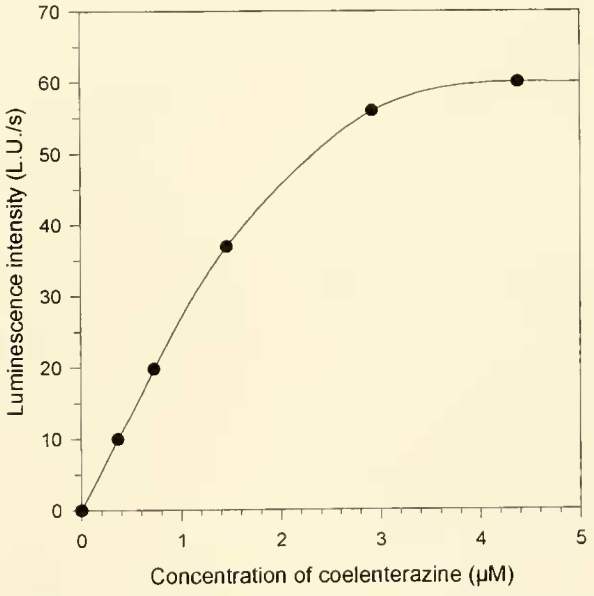

Figure 8. Effect of coelenterazine concentration on the intensity of luminescence when the reaction was catalyzed by *Periphylla* luciferase-L. The concentration of coelenterazine represents the final concentration.

estimated to be about 1.1 μM. The maximum specific activity (the specific activity measured in the presence of a saturating concentration, about 5 μM, of coelenterazine) of the purest luciferase-L preparation was 8.0×10^{13} quanta/mg · s, assuming an $A_{280\,nm,\,1\,cm}$ value of 1.0 for 1 mg/ml solution of this enzyme. For luciferase-O, the Michaelis constant and the maximum specific activity of luciferase-O were estimated to be 0.2 μM and 5.5×10^{12} quanta/mg · s, respectively (data not shown). The activity of luciferase-O is highly variable, dependent on the environment and the composition of buffer solution; the activity is almost certainly much greater in the insoluble form that originally existed in association with particulate matter. In any case, the values given above for specific activity must be considered as minimal values, because the luciferase preparations used were not completely pure. For comparison, the specific activities of *Renilla* luciferase (M_r 35,000; Matthews *et al.*, 1977) and *Oplophorus* luciferase (M_r 130,000; Shimomura *et al.*, 1978) under comparable conditions were 1.9×10^{13} quanta/mg · s and 1.34×10^{14} quanta/mg · s, respectively (Inouye and Shimomura, 1997).

A problem arose in the determination of the quantum yield of coelenterazine in the presence of luciferase-L. The purified luciferase-L apparently contained a trace impurity that inactivated coelenterazine by binding it tightly or by oxidizing it without light emission. This impurity eluted from the Superdex 200 column in the final step of purification (Fig. 1) immediately after the peak of luciferase; thus it could not be completely eliminated from the purified luciferase. The effect of this coelenterazine-inactivating impurity was reduced greatly, but not completely, by heating luciferase-L in the assay buffer at 100°C for 10 min. The complete elimination of the effect of this impurity was achieved only by pretreating the luciferase-L with coelenterazine. Thus, the quantum yield of coelenterazine was measured by repeated additions of a small amount of coelenterazine (5 μl of 0.2 mM methanolic solution) to a solution of luciferase-L; each new addition of coelenterazine was made only after the light emission from the previous addition had been completed. In this way, the total light emitted in each addition of coelenterazine successively increased, reaching a constant, maximum value after five or six additions. The quantum yield of coelenterazine calculated for the last addition was 0.14; this value was taken as the quantum yield of coelenterazine catalyzed by luciferase-L. Luciferase-O contained no significant inhibitor, and the quantum yield of coelenterazine catalyzed by luciferase-O was 0.12. For reference, the quantum yield of coelenterazine in the presence of *Renilla* luciferase was 0.069 (Hart *et al.*, 1979) and 0.11 (Inouye and Shimomura, 1997); the quantum yield in the presence of *Oplophorus* luciferase was 0.34 at 22°C (Shimomura *et al.*, 1978).

Inhibitors of the luminescence reaction

Periphylla luciferase was strongly inhibited in different fashions by two types of compounds, cupric salts and thiol compounds. The inhibition of coelenterazine luminescence by Cu^{2+} was instantaneous and very strong. The luminescence catalyzed by luciferase-L was inhibited by 25% with 10 μM Cu^{2+} and 85% with 30 μM Cu^{2+}; when catalyzed by luciferase-O, the inhibition was 70% with 1 μM Cu^{2+} and 97% with 10 μM Cu^{2+}. Various enzymes are inhibited by heavy metals, but *Periphylla* luciferase was inhibited by Cu^{2+} almost specifically; *i.e.*, much more strongly than by other metals (such as Ba^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+}). The significance of this finding is unknown.

The inhibition by thiols is not instantaneous. The loss in the activity of luciferase-L in the presence of 0.3 mM 2-mercaptoethanol was 60% after incubation for 20 h at 0°C; in the presence of 1 mM, the loss was 95%. With luciferase-O, under the same conditions (after 20 h at 0°C), the activity loss was 27% in the presence of 1 mM 2-mercaptoethanol and 92% in the presence of 10 mM. The inhibition was probably caused by the reduction of a functional S-S bond to thiol groups in the luciferase molecules. *Cypridina* luciferase is similarly inhibited by 2-mercaptoethanol (unpubl. data).

Significance of the existence of two different luciferases

To find two or more isoenzymes in a single species of organisms is not unusual. In the case of the hydrozoan jellyfish *Aequorea aequorea*, eight slightly different forms of the photoprotein aequorin have been isolated (Shimomura, 1986), although a photoprotein is not an enzyme in a strict sense. In the case of *Periphylla* luciferase, however, the designation of isoenzymes does not fit well for luciferase-L (M_r 32,000) and luciferase-O (M_r 75,000), because of the large difference in their molecular sizes. Based on their molecular weights, it is possible that luciferase-O is a derivative of luciferase-L; luciferase-O might be a dimer of luciferase-L or a conjugate of luciferase-L and an unrelated protein molecule.

As judged by their light emission, luciferase-L in live specimens of *P. periphylla* exists in photocytes scattered in the exumbrellar epithelium of the dome and lappet, whereas luciferase-O occurs in the cortical layer of eggs (Flood *et al.*, 1996). The bioluminescence response of the exumbrellar photocytes may have some practical functions—serving as a physiologically controlled defense mechanism, for example (Herring *et al.*, 1996); but the possible light emission from the eggs inside the ovaries of the adult medusae is difficult to rationalize. Although these ovaries and eggs are easily visible from below the medusa, light emission has never been observed in response to mechanical and electrical stimuli that readily elicit bioluminescence from the exumbrellar epithelium.

Only strong mechanical stimuli (rough rubbing) or strong osmotic shock (immersion in tap water), which is likely to disrupt the cell membrane, have proven effective in causing the eggs to luminesce (Flood, unpubl. data). The bioluminescence of eggs of certain hydromedusae, such as *Phialidium gregarium* and *Mitrocoma cellularia,* has been reported to rely on photoprotein systems (Freeman and Ridgway, 1987).

In the ovary of *Periphylla,* luciferase-O exists in an insoluble form bound to particulate matter; the enzyme is unusually stable and highly resistant to inactivation under various conditions. Indeed it is far more stable than luciferase-L, luciferase-O (soluble), *Oplophorus* luciferase, or *Renilla* luciferase, under all the conditions tested: heating at 90°C; adding guanidine hydrochloride, detergents, or organic solvents (methanol, ethanol, acetonitrile, etc); and leaving at room temperature for more than 2 weeks. The high stability of the insoluble form of luciferase-O, as well as its insolubility, might have evolved through an adaptation to the fat-rich ovarian environment, for the purpose of preserving the luciferase until oocytes begin to develop and stimulable bioluminescence becomes functional in early embryos.

Conclusions

The bioluminescence system of the scyphozoan jellyfish *Periphylla periphylla* was shown to be a coelenterazine-luciferase system; it is the first fully confirmed example in luminous jellyfishes. The *Periphylla* bioluminescence system is remarkable in two ways: First, the system involves two apparently different luciferases, luciferase-L and luciferase-O, that occur separately in two different types of cells in a single animal. Second, these luciferases are highly resistant to certain drastic processes of inactivation: luciferase-L withstands boiling at 100°C, and luciferase-O functions normally in denaturing environments containing 2 *M* guanidine hydrochloride. This high resistance to inactivation, however, is not indicative of a high stability at normal experimental conditions; the stabilities of luciferase solutions at neutral pH are similar to those of *Oplophorus* luciferase and *Renilla* luciferase when left for an extended period at room temperature (Shimomura, unpubl. data).

The heat resistance of luciferase-L is far greater than that of any luciferase previously known. Most other luciferases, including those of the luminous bacteria, the ostracod *Cypridina,* the limpet *Latia,* and the krill *Euphausia,* are inactivated rapidly and irreversibly between 30°C and 50°C; the most heat-resistant examples known previously are the partially purified preparations of *Oplophorus* luciferase, which withstand temperatures up to about 70°C (Shimomura *et al.,* 1978; Shimomura, 1995b). A structural study of *Periphylla* luciferase molecules, to clarify the cause of their unusual stabilities, together with studies of other luminous scyphozoans such as *Atolla* and *Pelagia,* would be important and interesting next problems.

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